Angling selects against active and stress-resilient phenotypes in rainbow trout

Barbara Koeck[1 *], Libor Závorka[1], David Aldvén[1], Joacim Näslund[1], Robert Arlinghaus[2,3], Per-Ove Thörnqvist[4], Svante Winberg[4], Björn Thrandur Björnsson[1] and Jörgen I. Johnsson[1]

[1] Department of Biological and Environmental Sciences, University of Gothenburg, Sweden

[2] Department of Biology and Ecology of Fishes, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany

[3] Division of Integrative Fisheries Management, Faculty of Life Sciences, Humboldt Universität zu Berlin, Germany

[4] Department of Neuroscience, Physiology Unit, Biomedical Center BMC, Uppsala University, Sweden

* corresponding author; e-mail: koeckbarbara@hotmail.com ; barbara.koeck@glasgow.ac.uk

Current affiliations:

Barbara Koeck: Institute of Biodiversity, Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, Graham Kerr Building, University of Glasgow, G12 8QQ, Glasgow, UK

Libor Závorka: Laboratoire Évolution and Diversité Biologique (EDB UMR 5174), CNRS, Université de Toulouse, Toulouse, France

Joacim Näslund: Department of Zoology, University of Stockholm, Sweden

Abstract

Selection induced by human harvest can lead to different patterns of phenotypic change than natural predation and could be a major driving force of evolution of wild populations. The vulnerability of individuals to angling depends on the individual decision to ingest the bait, possibly mediated by their neuroendocrine response towards the associated stimulus. To investigate the mechanisms behind individual vulnerability to angling, we conducted angling experiments in replicated ponds and quantified individual behavioral traits and neuroendocrine stress responsiveness in two salmonid species, rainbow trout and brown trout. We discovered a phenotypic syndrome in rainbow trout, but not in brown trout, where lower serotonergic and dopaminergic brain activity and cortisol levels (i.e., lower stress responsiveness) in response to a standardized experimental stressor were associated with higher activity, forming a proactive phenotype that showed increased vulnerability to angling. Our results show that angling targets the most stress-resilient and active phenotypes of rainbow trout, supporting the suggestion that fishing-induced phenotypic selection may lead to an increased representation of stress-responsive and low-activity phenotypes in harvested populations.

Keywords: coping styles, fishing selection, hook avoidance, pace-of-life syndrome, phenotypic integration

Introduction

Natural and human predation often select for different traits, which can lead to divergent or even opposing selection patterns (Carlson et al. 2007). For example, while natural predators are usually gape-limited (Godin 1997), fisheries tend to focus on the harvest of the largest individuals of the population because of their value and physical gear constraints, and facilitated by harvest regulations (Jørgensen and Holt 2013; Allendorf and Hard 2009). A large body of literature has revealed that intense and size-selective harvesting favors evolution of fast life-history (e.g., Jørgensen et al. 2009; Heino et al. 2015; Uusi-Heikkilä et al. 2015). The pace-of-life syndrome (POLS) hypothesis predicts that individual life-history, behavioral and physiological traits are correlated along a gradient of slow to fast life-history strategies (Réale et al. 2010). Accordingly, individuals with for example a fast pace-of-life are predicted to be bold and active, and to have a low hypothalamic-pituitary-adrenal (HPA) axis reactivity, i.e. low stress responsiveness, traits associated with rapid growth (Koolhaas et al. 1999; Coppens et al. 2010). Following the POLS, fishing selection might in turn also lead to corresponding changes in the behavior and stress responsiveness of surviving phenotypes (Biro and Post 2008; Uusi-Heikkilä et al. 2008). Evidence for fisheries-induced evolution of behavior and physiology is however scarce (Heino et al. 2015; Arlinghaus et al. 2017; Díaz Pauli and Sih 2017; Hollins et al. 2018).

Furthermore, with passive fishing gear, the harvest-induced selection depends on the ultimate decision of an individual fish to ingest a bait, attack a lure or approach a trap or gillnet, based on the perception and processing of different sensory cues (Andersen et al. 2016; Arlinghaus et al. 2017; Hollins et al. 2018). A number of studies have shown that vulnerability to harvest by passive fishing gear, such as angling, is positively related to behavioral traits such as boldness, aggression, exploration or activity (Klefoth et al. 2012;

65 Sutter et al. 2012; Härkönen et al. 2015; Wilson et al. 2015; Alós et al. 2016; Klefoth et al.
66 2017; Monk and Arlinghaus 2017a). However, the evidence is far from conclusive as several
67 empirical studies report no or limited correlations between angling vulnerability and certain
68 behavioral traits (Kekäläinen et al. 2014; Vainikka et al. 2016; Monk and Arlinghaus 2017b).

69 The pathways to a decision (i.e. to a behavioural response) varies between individuals
70 based on their global bodily state, defined by a combination of sensory information,
71 physiological and developmental state, and motivations (e.g., hunger or threat levels; LeDoux
72 2012). The behavioral response can therefore differ between individuals, mediated by
73 underlying differences in the activation of the hypothalamic-pituitary-interrenal axis (HPI
74 axis) that controls the release of corticosteroids to the blood circulation (e.g., cortisol), and
75 brain neurochemistry (e.g., serotonin and dopamine monoamine neurotransmitters and
76 noradrenalin; Andersen et al. 2016; Coppens et al. 2010; Wendelaar Bonga 1997). While
77 evidence has been provided revealing a link between stress responsiveness (i.e. activation of
78 the HPI-axis) and cognitive appraisal of aversive stimuli (Moltesen et al. 2016), this link has
79 rarely been investigated in the context of vulnerability to fishing (Louison et al. 2017) and
80 remains not well understood (Hollins et al. 2018).

81 The divergent results found in studies investigating the behavioral drivers for
82 vulnerability to fishing might arise from gear and species-specific differences and be affected
83 by the context of studies. For instance, compared to studies in controlled laboratory
84 environments, in natural systems the spatial component might be a more important driver for
85 the encounter between gear and fish overriding the individual variability in the decision
86 pathways to attack a lure or ingest a bait (Monk and Arlinghaus 2017a,b). To improve our
87 understanding of the mechanisms that drive the capture process and identify which traits
88 might be under selection, studies on populations with a known phenotypic distribution

conducted at relevant spatio-temporal scales that allow fish to express their natural response to fishing gear (Klefoth et al. 2012; Härkönen et al. 2014) are required. Such studies can then be scaled to population-level processes through whole system studies or modelling (Andersen et al. 2018).

In the present study, we investigated, using a replicated pond system, the selection mechanisms of angling by looking at the behavioral and neurobiological basis of the variability in vulnerability to angling in two salmonid species with different domestication histories, rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) and brown trout (*Salmo trutta*, L.). Specifically, we addressed the following questions: (i) Do capture patterns differ between rainbow trout and brown trout? (ii) Are behavioural type and stress responsiveness correlated phenotypic traits? (iii) Is vulnerability to angling linked to individual phenotype of fish?

Material and Methods

Experimental setup and activity monitoring

To answer our questions, we used four semi-controlled mesocosm ponds to carry out two replicated angling experiments on stocked fish of known size distribution and for which we recorded capture rates, order of capture, individual activity and stress responsiveness. Each mesocosm pond (area = 720 m^2, depth = 2 m), belonging to the Swedish Anglers' Association (*Sportfiskarna*) in Gothenburg, Sweden (57.693°N, 12.037°E; Appendix A1), was stocked with size-matched rainbow (298 ± 19 mm) and brown trout (281 ± 15 mm) in equal densities (25 individuals per species and pond). Both trout species originated from the same hatchery and were reared under comparable conditions (*Källefalls Fiskodling*). The brown trout were F1 offspring from wild parents captured in the near-by *Lake Vättern*. The

112 rainbow trout were of a domesticated strain, bred since 1997 within the hatchery and used
113 exclusively for stocking in Swedish waters.

114 Prior to release, the fish were anesthetized (10 % benzocaine at 0.3 ml L^{-1}), measured for
115 body wet mass and length, and a passive integrated transponder (PIT-tag; HDX ISO
116 11784/11785, 23 mm, 0.6 g in air, Texas Instruments Inc.) was inserted into the coelomic
117 cavity for individual identification and activity monitoring in the ponds (thereafter, referred
118 to as pond activity). A custom-made radio frequency identification detection (RFID) system
119 was deployed in each pond to monitor pond activity of tagged fish, which was recorded over
120 35 days, covering a period before, during and after the angling experiments (between October
121 2nd and November 4th, 2014). Each RFID system consisted of a set of four detection antennas
122 (2.2 × 2.3 m) deployed vertically and connected to a RFID half-duplex reader (Oregon
123 RFID), which recorded time and date, antenna ID, and the PIT tag code for each detected
124 fish. Detection range of antennas covered the entire antenna surface within a distance of 50
125 cm around the antenna frame. Raw detection data from the RFID antenna monitoring system
126 was filtered based on fish ID, detection interval and antenna, in order to account for the bias
127 induced by repeated detections of a single fish when remaining in the vicinity of the detection
128 array of an antenna. The filtered number of detections per individual was highly correlated to
129 the number of relocations between two antennas (*i.e.* number of times a fish moves between
130 two antenna frames at distinct positions; Spearman rank correlation: $r = 0.88$, $p < 0.001$). This
131 indicated that the filtered number of detections of our RFID system can be used as a reliable
132 proxy for individual activity, telling apart inactive fish from moving fish, and is not inflated
133 by the detections of stationary fish nearby an antenna.

134 **Angling treatments**

Two common types of angling techniques were used; natural shrimp baits on a single hook and spinners as example of an artificial lure. Natural bait-angling was carried out using the shrimp on a barbless hook attached beneath a float. Anglers were fishing passively with the shrimp bait by casting it and retrieving it slowly, while lure angling was fished actively using a spinner (Myran WIPP Yellow/White, 10 g), which was cast and retrieved at a faster speed. With the exception of these two types of terminal tackles, identical angling equipment was used for the experiments (Appendix A2). Angling was carried out by instructed and experienced volunteer anglers. Hooked fish were landed with a knotless net, unhooked and kept in a holding tank until returned to their pond at the end of the fishing event (i.e., day). A fish could thus be captured only once per fishing event (i.e., day), but recaptured at each new event in following days.

Experiment 1. After 26 days of acclimation in the experimental ponds, including 10 days of pre-angling activity monitoring, the first fishing experiment was conducted. Fishing experiments were designed to ensure a certain level of fish captures despite the small-scale study system and limited experimental period. It consisted of a total of 10 days of angling, with five days (i.e. events) of natural bait angling followed by five days of lure angling, with two angling-free days in between. Each angling day, one angling event of one hour of effective angling time took place at dawn, during which three anglers were rotating every tenth minute within or between ponds according to a randomization schedule to control for bias in fishing skills of anglers and site preference (Appendix A2). Overall, each pond received a total angling effort of 30 h (3 anglers x 1 hour x 5 days x 2 weeks) or 42 angler hours per hectare. In experiment 1, angling was practiced in only two of the four experimental ponds (pond 1 and 3; Fig. A1) and was designed to identify (i) the individual vulnerability to angling over time and its phenotypic correlates in rainbow trout and in brown trout, and (ii) the species-specific selectivity of angling technique.

160 ***Experiment 2.*** The second angling experiment consisted of three angling events with
161 simultaneous bait and lure angling and a total angling effort of 6 hours per pond (2 anglers ×
162 1 hour × 3 days; 8.5 angler hours per hectare) or following the same procedure as in
163 experiment 1 (see also Appendix A2). Angling was this time performed in all four ponds (see
164 Fig. A1), to compare hook avoidance response in fish that have either been previously
165 exposed or that were naïve to angling. Both angling techniques were used simultaneously to
166 verify the results from the species-specific selectivity of angling technique from experiment
167 1, while excluding the temporal effect of a successive use of angling technique.

168 **Stress responsiveness to a standard stressor**

169 After the angling experiments, we measured individual stress-responsiveness of fish by
170 quantifying the products of the two main hormonal systems involved in stress response in fish
171 (i.e. corticosteroids, catecholamines and their monoamine precursors; Schreck and Tort 2016)
172 in response to a standardized exposure to an experimental stressor (i.e. transfer to holding
173 tanks for a minimum of 12h + 30 min open-field test). To do so, we drained the ponds and
174 transferred fish to flow-through holding tanks where we kept them for minimum 12 h before
175 exposing them to a 30 min open-field test (see Appendix A3 for detailed protocol of the
176 open-field test). Following the behavioral scoring, we anaesthetized fish (10 % benzocaine at
177 0.5 ml L^{-1}) and measured their body mass and length (SL) to get a measure of growth, before
178 we assessed their individual physiological stress responsiveness by measuring circulating
179 plasma cortisol, brain neurotransmitter activity (serotonin and dopamine system,
180 noradrenaline).

181 Blood samples were taken from the caudal vein into a 1-ml heparinized syringe and kept
182 on ice until sampling of a group of four fish was completed. Blood was then centrifuged for 7
183 min, plasma obtained, frozen on dry ice and stored at -80 °C until analysis. Plasma cortisol

levels were measured by radioimmunoassay (Young 1986; Sundh et al. 2011). Anaesthetized fish where then euthanized by cutting the spinal cord. Brains were dissected out and separated into hindbrain (optic tectum, cerebellum and brain stem) and forebrain (telencephalon and diencephalon), frozen in tinfoil on dry ice and stored at -80 °C until analysis. Monoamine neurotransmitters and metabolites were extracted from the hindbrain and measured using HPLC-ED (high performance liquid chromatography with electrochemical detection). Levels of noradrenaline (NA), serotonin (5-hydroxytryptamine, 5-HT), dopamine (DA), and DA and 5-HT major metabolites (5-hydroxyindoleacetic acid (5-HIAA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were measured after being normalized for wet mass of brain tissue and expressed in ng by g of brain. The ratios between metabolite and monoamine, 5-HIAA/5-HT and DOPAC/DA, were used as indicators for serotonergic and dopaminergic activity, respectively.

Data analysis

Overall and species-specific catchability. We modeled the effects of species, angling gear and previous exposure to angling on number of captures collected from the two angling experiments using generalized linear models (GLM; using a Poisson distribution with log link-function). Using angling experiment 1, we compared the number of captures using bait angling between species (i.e. rainbow trout, brown trout) and the number of captures using artificial lure between species. In order to get an idea of general hook avoidance, we used the capture data from experiment 2 to compare the number of captures based on previous exposure to angling, i.e. from ponds 2 and 4, where fish were naïve to angling with those from ponds 1 and 3, where fish had already been exposed to angling. We then used the data from only ponds 2 and 4, with fish that were naïve to angling, to compare the number of captures by species and fishing gear (i.e. natural bait, artificial lure). No significant pond, or size effect were found and were thus removed from the final models.

Association between phenotypic traits. Correlations between activity measured in the ponds, and the physiological variables involved in a stress response were tested using Pearson's correlation test, estimating Pearson's *r* and its 95% Confidence Intervals (CI). Adjusted *p*-values were computed using the false discovery rate method to account for multiple comparisons (Benjamini and Hochberg 1995). Data analysis was done separately for rainbow and brown trout. A further distinction was made for fish from ponds 1 and 3 and fish from ponds 2 and 4 as they were exposed to different total angling efforts, which may have influenced physiological stress response of fish measured after the experimental angling. Pond 1 and 3 are thereafter referred to as high angling intensity treatment (36 hours of angling effort or 42 angler hours per hectare), and pond 2 and 4 as low angling intensity treatment (6 hours of angling effort or 8.5 angler hours per hectare). Angling intensities from both treatments can be compared to angling efforts from small lakes or put-and-take ponds over the course of a few days to a couple of weeks. Correlation analysis showed that pond activity and physiological measurements were not related to body size nor to specific growth rate (SGR). In general, fish presented a slight loss in wet body mass between the beginning and end of the experiment, and a low variability of SGR between individual fish (mean ± SD rainbow trout: SGR=−0.18±0.07, brown trout: SGR=−0.16±0.06). Accounting for individual fish size or growth was therefore deemed unnecessary for subsequent analysis.

Vulnerability of fish to angling and phenotypic correlates. The vulnerability of individual fish to capture by angling was modeled using semi-parametric Cox proportional hazard regressions, with the probability until first capture as response variable. Comparisons were first made between species, and then for each species a separate model was constructed to investigate the relationship with individual plasma cortisol levels (in response to a standard experimental stressor), activity monitored in the ponds and activity monitored in laboratory conditions. Activity recorded in laboratory open-field test was however unrelated to

vulnerability to angling in both species and could thus be removed from the survival models. Such survival analysis enables to incorporate the information of captured and uncaptured individuals (i.e. right censored data), as well as of the time to event (e.g. the capture order of individual fish). For the single species model, the used hazard function is of the form:

$$h(t|z) = h_0(t)\exp(\beta\, z)$$

where h_0 is the baseline hazard, z is a time independent predictor (i.e. activity in the ponds or in laboratory conditions or plasma cortisol) and β is the hazard coefficient, estimated using a partial likelihood function.

At the end of the experimental period, some brown trout males were maturing and were excluded from data analysis to avoid any confounding effects related to maturation. This explains the slightly lower sample sizes for the analyses of brown trout compared to rainbow trout data (see results section for details). Data handling and analysis was computed using the packages *car* and *survival* for the *R* statistical environment.

Results

Technique- and species-specific vulnerability to angling

In experiment 1, 72 out of 100 fish (72 %) were captured at least once. Overall, more rainbow trout were captured (45 out of 50 fish, i.e. 90 %), than brown trout (27 out of 50 fish, i.e. 54 %; Fig. 1). The time to capture and the probability of remaining uncaptured was significantly lower in rainbow trout than in brown trout (see results from the survival analysis Table 1; Fig. 2), indicating a lower intrinsic vulnerability to angling and/or stronger hook avoidance in brown trout. More recaptured individuals and higher individual recapture rates were observed in rainbow trout (25 fish recaptured, up to 3 times and one fish up to 4 times over the two experiments) compared to brown trout (9 fish recaptured only once). Additionally, in

256 experiment 2, the number of fish captured in ponds previously exposed to angling was
257 significantly lower than in ponds with fish naïve to angling (effect of angling treatment: $F_{1,25}$
258 = 5.12, p = 0.032; Fig. 3), indicating an avoidance response in both fish species when
259 previously exposed to angling.

260 During the first week of experiment 1 using natural bait, significantly more rainbow trout
261 than brown trout were captured ($F_{1,16}$ = 11.08, p = 0.004; Fig. 1), whereas no significant
262 differences were observed between rainbow trout and brown trout captured during the second
263 week with spinner lures ($F_{1,16}$ = 1.61, p = 0.223; Fig. 1). This result was confirmed in
264 experiment 2, with simultaneous bait and lure angling (interaction of species and angling
265 technique effect: $F_{1,22}$ = 5.71, p = 0.026): more rainbow trout were captured than brown trout
266 with natural bait (Tukey post-hoc test, Z = -2.75, p = 0.020; Fig. 3), but there was no species
267 difference in relation to captures with lures (Tukey post-hoc test, Z = 0.27, p = 0.989; Fig. 3).

268 **Context-dependency of phenotypic associations between behaviour and neuroendocrine**
269 **stress response.**
270 For rainbow trout from the high intensity angling treatment (n = 45; Fig. A2; Fig. 4), cortisol
271 levels, 5-HIAA, and DA turnover (DOPAC/DA) were all positively correlated to each other
272 and negatively correlated with pond activity. Additionally, cortisol, 5-HIAA and 5-HT were
273 positively correlated with DOPAC. For brown trout from the high intensity angling treatment
274 (n = 35), 5-HIAA and 5-HT were positively correlated with DOPAC and DA turnover (Fig.
275 A4), and pond activity was correlated to 5-HIAA, 5-HT and 5-HT turnover. Associations
276 between levels of monoamine neurotransmitters in rainbow trout and in brown trout, in
277 response to a standard experimental stressor, indicated the presence of inter-individual
278 differences in the general activation of the HPI-axis in both species. Our results further

indicate an association between swimming activity in the ponds and activation of the HPI-axis in both species.

For rainbow trout from the low intensity angling treatment (n = 43) however, no correlations between cortisol, monoaminergic activity nor pond activity were visible (Fig. A3). For brown trout from the low intensity angling treatment (n = 35), 5-HT was positively correlated with DA and activity (Fig. A5). Overall, the results showed that phenotypic associations were mainly revealed under a context of high and repeated angling intensity and were less evident for fish from the low intensity angling treatment.

Vulnerability of fish to angling and phenotypic correlates

Results from angling experiment 1 (pond 1 and 3) showed that rainbow trout that were caught first and less likely to remain uncaptured over the course of the experiment had a higher pond activity and a weaker cortisol response (Table 2, Fig. 5a), indicating that the most active and stress responsive rainbow trout were relatively more vulnerable to angling. In brown trout, for which catch rates were much lower than for rainbow trout, no such relationship was found (Table 3, Fig. 5b).

Discussion

Species selection: conditioned hook avoidance and potential effect of domestication

At a species level, our study confirmed that rainbow trout are more vulnerable to angling than brown trout. This finding is consistent with previous angling experiments conducted in both rivers and ponds (Anderson and Nehring 1984; Pawson 1991; Mezzera and Largiadèr 2001) and thus seems to be a general among-species pattern. But we also found that the relative vulnerability to angling between both species was technique-specific: whereas rainbow trout was more vulnerable to natural bait angling than brown trout, both species were equally vulnerable to lure angling. Studies on a range of predatory species suggest that catchability is often higher for natural bait compared to artificial lures (Beukema 1970; Härkönen et al. 2015; Moraga et al. 2015), indicating that when associated with a reward (i.e. natural bait) the conditioned avoidance in response to the aversive stimulus related to angling is reduced. These findings were mirrored in our work, where catch rates were overall and specifically for rainbow trout higher for natural bait than for artificial lures. Hook avoidance of artificial lures was generally extremely rapid within just one or two fishing days, similar to findings in other studies (e.g., Askey et al. 2006). These species-specific findings indicate that both species respond differently to the cues associated to the passively fished natural bait, shaping the relative vulnerability of both species. It is likely that the mechanisms underlying the effects relate to interspecific behavioral and physiological differences, and may additionally be shaped by domestication effects.

Numerous studies have highlighted behavioral and physiological intraspecific differences between wild and domesticated strains, the later ones being recognized to be bolder and more risk-prone (Johnsson et al. 2001; Sundström et al. 2004; Huntingford and Adams 2005), and presenting weaker neuroendocrine stress responsiveness than wild genotypes (i.e. lower dopaminergic turnover DOPAC/DA and serotonergic turnover 5-HIAA/5-HT, due to lower

levels of serotonin 5-HT and dopamine DA; Lepage et al. 2000). Both species used in this experiment were hatchery-reared, but rainbow trout came from a strongly domesticated strain shaped by decades of selective breeding whereas the brown trout stemmed from wild parents. Although in our experiment, species and degree of domestication is confounded, it is possible that the long domestication history rainbow trout were exposed to contributed to their higher intrinsic vulnerability, as shown before for carp (*Cyprinus carpio* L.) genotypes differing by degree of domestication (Klefoth et al. 2012). Whereas brown trout and rainbow trout differ in terms of stress physiology (e.g. rainbow trout has a faster recovery in terms of primary and secondary stress response compared to brown trout; Ruane et al. 1999) and behavior (e.g foraging and habitat use; Blanchet et al. 2007), the observed increased angling vulnerability of rainbow trout relative to brown trout can thus also be partly attributed to effects of domestication. Existing studies show indeed that wild strains of rainbow trout have higher survival rates and are less likely to be harvested by angling than domesticated strains (Brauhn and Kincaid 1982; Dwyer and Piper 1984).

Activity and stress responsiveness explain variability in vulnerability to angling in rainbow trout

As shown in previous studies, the capture data from our angling experiments has highlighted the presence of individual differences in vulnerability of fish to angling shaping overall population-level catchability (e.g., Beukema 1970; Askey et al. 2006). Such differences were particularly marked in rainbow trout for which certain individuals had been recaptured up to four times while others remained uncaptured despite repeated angling effort. In brown trout, capture rates declined rapidly after one day of fishing and remained overall much lower than for rainbow trout. Previous studies using selection lines of largemouth bass (*Micropertus salmoides*) of high vs. low vulnerability to angling have highlighted that differences in susceptibility to capture can be linked to individual variability of physiological traits such as

344 metabolism and anaerobic activity (Redpath et al. 2010), but also of levels of circulating
345 cortisol as indicator for stress responsivness of fish (Louison et al. 2017). In our experiment,
346 after the standardized exposure to an experimental stressor, both species showed generally
347 elevated cortisol levels, which were about 10-fold higher than basal levels reported for fish
348 (Pankhurst 2011), indicating that all fish were in a stressed state when brain tissues were
349 sampled to measure neurotransmitter activity. However, individual variability in levels of
350 corticosteroids, catecholamines and their monoamine precursors were also evident in our
351 results indicating differences in stress responsiveness of individual fish within species.
352 In rainbow trout that had been repeatedly exposed to angling (i.e. high intensity
353 angling treatment), we discovered a phenotypic syndrome, i.e. co-variation among
354 functionally related traits that defines how the organism interacts with its environment and
355 sustains itself (Pigliucci and Hayden 2001; Závorka et al. 2017). Individual pond activity and
356 neuroendocrine activity (i.e. dopamine turnover and 5-HIAA, a major metabolite of
357 serotonin) and cortisol response (after exposure to a standardized experimental stressor) were
358 associated and correlated with individual vulnerability to angling, such that active rainbow
359 trout had a relative weaker neuroendocrine stress responsiveness than less active rainbow
360 trout. The role of neurotransmitters in stress physiology and behavioral plasticity in
361 vertebrates (Coppens et al. 2010), including fish (Winberg and Thörnqvist 2016), has recently
362 been highlighted. The chronic activation of the brain serotonergic system can cause
363 behavioral inhibition of feeding, locomotion and aggression (Winberg and Thörnqvist 2016).
364 Furthermore, the dopamine system is known to have a central role in conditioned behavioral
365 responses, influencing the motivational control over behavior (Wise 2004) affecting for
366 instance risk-taking or avoidance behavior (Höglund et al. 2005; Arias-Carrion and Poeppel
367 2007). Dopamine is also the precursor of noradrenaline, known to increases arousal and
368 general alertness (Singh et al. 2015). In rainbow trout, individual differences in the activation

of the HPI axis could therefore explain the observed differences in their behavior towards fishing gear, via possible mechanism of sensory modulation leading to behavioral habituation or inhibition, shaping the hook avoidance response under repeated angling stimuli.

The phenotypic syndrome associating pond activity and physiological stress responsiveness (association with the activation of the serotonergic system for brown trout and additionally with the activation of the dopamine system and levels of circulating plasma cortisol in rainbow trout) was however only visible under high angling intensity. In particular, in rainbow trout previously exposed to a low angling intensity there was a total absence of associations of physiological stress responsiveness and behavioral traits. Similarly, Killen and colleagues (2013) have shown that the relationships between behavioral and physiological traits are sometimes only revealed by the presence of an environmental stressor. We suggest that the phenotypic syndrome linking behavioral and physiological traits (i.e. stress response) in rainbow trout was revealed only under repeated angling causing a chronic stress response. Furthermore, it has been shown in rainbow trout, that only the chronic activation of the brain serotonergic system causes a general behavioral inhibition (Winberg et al. 2001; Winberg and Thörnqvist 2016).

For brown trout, while we also noted an association between pond activity and serotonergic activity, we found no link between vulnerability to angling and the measured phenotypic traits. This could be simply due to the overall lower catch rates found in brown trout compared to rainbow trout, and explained by the fact that brown trout recovers slower in terms of primary and secondary stress response in comparison to rainbow trout (Ruane et al. 1999), therefore increasing hook avoidance in that species. Additionally, sexual maturity was observed in some brown trout at the end of the experiment and might have affected their stress responsiveness (Pottinger et al. 1995). Further experiments, allowing longer recovery periods between angling events or applying lower fishing effort that is more realistic of

natural settings, are required to investigate the behavioral, physiological and neurobiological mechanisms of angling selection in brown trout and wild fish in general.

Management implications

The results we found provide empirical evidence that harvest by angling can selectively target a non-random subset of harvested populations, characterized by active and stress-resilient phenotypes and that this result is not necessarily applying across contexts and species. However, for some species based on the documented assumption that the variation of phenotypic traits presents some degree of heritability (Danchin et al. 2011; Philipp et al. 2009), the selective removal of the most stress resilient and active phenotypes by intensive angling may cause a shift towards an increase of shy phenotypes in exploited compared to unexploited populations (Arlinghaus et al. 2017; Louison et al. 2017).

The progressive removal of the fraction of highly vulnerable individuals can then generate a decrease in catchability (Alós et al. 2016) and affect the size-selectivity of harvesting (Tsuboi et al. 2016), ultimately affecting the satisfaction of anglers (Arlinghaus et al. 2017). In catch-and-release fisheries, even if fish populations might remain unaffected by evolutionary effects linked to selective removal, behavioral plasticity or adaptation in response to direct and indirect exposure to fishing gear can further decrease the fishing quality and catchability (Askey et al. 2006). In fact, in comparison to consumptive fisheries, under catch-and-release regulations fish have the potential to learn to identify and avoid the threat associated to angling via mechanisms of private (Beukema 1970; Klefoth et al. 2013), but also public information use (Danchin et al. 2004). This suggests that under intensive angling, fishing success will increasingly become density-independent. Rotational management systems might then be required in small-scale fisheries with high fishing pressure to enable fish to return to vulnerable stages after direct or indirect exposure to angling (Camp et al. 2015).

418 Domesticated fish appear generally to be more vulnerable to capture than wild fish
419 (Brauhn and Kincaid 1982; Dwyer and Piper 1984; García-Marín et al. 1998; Mezzera and
420 Largiadèr 2001; Biro and Post 2008). Our study adds to this literature showing that angling
421 within a domesticated strain targets the most stress-resilient and active phenotypes,
422 considered to be the fraction of a population with the highest invasion potential (Phillips and
423 Suarez 2012; Juette et al. 2014). This indicates that angling could be tailored to reduce the
424 invasion potential of escaped or unintentionally released fish by reducing the propagule
425 pressure of the most invasive phenotypes, at least when applied shortly after the release and
426 in the vicinity of the release site. In practice, caution needs however to be taken as the
427 efficiency of angling will be diluted in large fishery systems and with time after release due
428 to behavioral plasticity and adaptation. Surviving domesticated fish can learn to avoid areas
429 exposed to angling pressure and adapt their behavioral response to environmental and human-
430 induced stressors rendering them less vulnerable to harvest over time, which may have
431 further potential cascading effects on ecosystem functioning (Evangelista et al. 2017;
432 Závorka et al. in press). Therefore, our study shall not be misread to suggest that angling
433 alone will be an efficient removal method of invasive phenotypes or species (e.g. Paul et al.
434 2003; Evangelista et al. 2015).

435 Results from our study show that angling targets the most stress-resilient and active
436 phenotypes of rainbow trout, supporting the suggestion that fishing-induced phenotypic
437 selection may lead to an increased representation of stress-responsive and low-activity
438 phenotypes in intensively harvested populations. Such selective removal may have further
439 potential spillover effects at higher levels of ecological organization (Palkovacs et al. 2018)
440 as well as for stock assessments and fisheries management (Arlinghaus et al. 2017).
441 However, as we did not find similar results in brown trout, we consider that further
442 experiments on wild fish and across environmental and fishing contexts are needed to

443 improve our understanding of fishing selection and predict possible effects of fisheries-
444 induced evolution. Such experiments should particularly focus on further investigating the
445 mechanistic link between behavioral and physiological traits that can possibly influence the
446 fishing process (Hollins et al. 2018).

Ethical Statement

These experiments were conducted under license 15-2014 issued by the Ethical Committee for Animal Research in Gothenburg, and comply with Swedish and European law.

Acknowledgements

We warmly thank the volunteer anglers and Per-Erik Jacobsen, Hans Lindqwist and Markus Lundgren from the Swedish Anglers Association *Sportfiskarna*. Many thanks to Jes Dolby, for his assistance with the RFID antennas, and Johan Höjesjö and Julien Cucherousset for their input and help at an earlier stage of this study. We also thank two anonymous reviewers for their excellent feedback. This study was part of the *SalmoInvade* project "Causes and consequences of invasions of aquatic ecosystems by non-native salmonids", funded by the *BiodivERsA* grant for research and innovation 2012-2013 from the European Union and the Swedish research council *FORMAS*. JIJ was also financed by the Interreg-project MarGen.

Data Accessibility

The data supporting this article has been posted to figshare
http://dx.doi.org/10.6084/m9.figshare.c.3467493

Author Contribution

BK, RA, JIJ developed the experimental design; BK, LZ, DA, JN carried out the experiment; POT, SV, BTB analyzed the samples; BK performed data analysis and wrote the initial draft; All authors contributed to the interpretation of results, the writing and editing of the paper.

References

Allendorf, F.W., and Hard, J.J. 2009. Human-induced evolution caused by unnatural selection through harvest of wild animals. Proc. Natl. Acad. Sci. U.S.A. **106**: 9987-9994.

Alós, J., Palmer, M., Rosselló, R., and Arlinghaus, R. 2016. Fast and behavior-selective exploitation of a marine fish targeted by anglers. Sci. Rep. **6**: 38093.

Andersen, B.S., Jørgensen, C., Eliassen, S., and Giske, J. 2016. The proximate architecture for decision-making in fish. Fish Fish. **17**: 680-695.

Andersen, K. H., Marty, L., and Arlinghaus, R., 2018. Evolution of boldness and life-history in response to selective harvesting. Can. J. Fish. Aqu. Sci. doi: 10.119/cjfas-2016-0350

Anderson, R.M., and Nehring, R.B. 1984. Effects of a catch-and-release regulation on a wild trout population in Colorado and its acceptance by anglers. N. Am. J. Fish. Manag. **4**: 257-265.

Arias-Carrion, O., and Poeppel, E. 2007. Dopamine, learning, and reward-seeking behavior. Acta Neurobiol. Exp. **67**: 481-488.

Arlinghaus, R., Laskowski, K.L., Alós, J., Klefoth, T., Monk, C.T., Nakayama, S., and Schröder, A. 2017. Passive gear-induced timidity syndrome in wild fish populations and its potential ecological and managerial implications. Fish Fish. **18**: 360-373.

Askey, P.J., Richards, S.A., Post, J.R., and Parkinson, E.A. 2006. Linking angling catch rates and fish learning under catch-and-release regulations. N. Am. J. Fish. Manag. **26**: 1020-1029.

Benjamini, Y., and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. Series B Meth. **57**: 289-300.

491 Beukema, J.J. 1970. Acquired hook-avoidance in the pike *Esox lucius* L. fished with artificial
492 and natural baits. J. Fish Biol. **2**: 155-160.
493 Biro, P.A., and Post, J.R. 2008. Rapid depletion of genotypes with fast growth and bold
494 personality traits from harvested fish populations. Proc. Natl. Acad. Sci. U.S.A.**105**:
495 2919-2922.
496 Blanchet, S., Loot, G., Grenouillet, G., Brosse, S. 2007. Competitive interactions between
497 native and exotic salmonids: a combined field and laboratory demonstration. Ecol.
498 Freshw. Fish **16**:133–143.
499 Brauhn, J.L., and Kincaid, H. 1982. Survival, growth, and catchability of rainbow trout of
500 four strains. N. Am. J. Fish. Manag. **2**:1-10.
501 Camp, E.V., van Poorten, B.T., and Walters, C.J. 2015. Evaluating short openings as a
502 management tool to maximize catch-related utility in catch-and-release fisheries. N.
503 Am. J. Fish. Manag. **35**:1106-1120.
504 Carlson, S.M., Edeline, E., Vollestad, L.A., Haugen, T.O., Winfield, I.J., Fletcher, J.M.,
505 James, J.B., and Stenseth, N.C. 2007. Four decades of opposing natural and human-
506 induced artificial selection acting on Windermere pike (*Esox lucius*). Ecol. Lett. **10**:
507 512-521.
508 Coppens, C.M., de Boer, S.F., and Koolhaas, J.M. 2010. Coping styles and behavioural
509 flexibility: towards underlying mechanisms. Philos. Trans. R. Soc. Lond., B, Biol.
510 Sci. **365**: 4021-4028.
511 Danchin, É., Charmantier, A., Champagne, F.A., Mesoudi, A., Pujol, B., and Blanchet, S.
512 2011. Beyond DNA: integrating inclusive inheritance into an extended theory of
513 evolution. Nat. Rev. Genet. **12**: 475-486.
514 Danchin, É., Giraldeau, L.-A., Valone, T.J., and Wagner, R.H. 2004. Public information:
515 from nosy neighbors to cultural evolution. Science **305**: 487.

516 Díaz Pauli, B., and Sih, A. 2017. Behavioral responses to human-induced change: Why
517 fishing should not be ignored. Evol. Appl. **10**: 231-240.
518 Dwyer, W.P., and Piper, R.G. 1984. Three-year hatchery and field evaluation of four strains
519 of rainbow trout. N. Am. J. Fish. Manag. **4**: 216-221.
520 Evangelista, C., Britton, R.J., and Cucherousset, J. 2015. Impacts of invasive fish removal
521 through angling on population characteristics and juvenile growth rate. Ecol Evol **5**:
522 2193-2202.
523 Evangelista, C., Lecerf, A., Britton, R., Cucherousset, J. 2017. Resource composition
524 mediates the effects of intraspecific variability in nutrient recycling on ecosystem
525 processes. Oikos **126**: 1439–1450.
526 García-Marín, J.L., Sanz, N., and Pla, C. 1998. Proportions of native and introduced brown
527 trout in adjacent fished and unfished Spanish rivers. Cons. Biol. **12**: 313-319.
528 Godin, J.-G. J. 1997. Evading predators. *In* Behavioural ecology of teleost fishes. *Edited by*
529 J.-G. J. Godin. Oxford University Press, Oxford, UK. pp 191–236.
530 Härkönen, L., Hyvärinen, P., Niemelä, P., and Vainikka, A. 2015. Behavioural variation in
531 Eurasian perch populations with respect to relative catchability. Acta Ethol. **19**: 21-31.
532 Härkönen, L., Hyvärinen, P., Paappanen, J., and Vainikka, A. 2014. Explorative behavior
533 increases vulnerability to angling in hatchery-reared brown trout (*Salmo trutta*). Can.
534 J. Fish. Aqu. Sci. **71**: 1900-1909.
535 Heino, M., Díaz Pauli, B., and Dieckmann, U. 2015. Fisheries-induced evolution. Annual
536 Rev Ecol. Evol. Syst. **46**: 461-480.
537 Höglund, E., Weltzien, F.-A., Schjolden, J., Winberg, S., Ursin, H., and Døving, K.B. 2005.
538 Avoidance behavior and brain monoamines in fish. Brain Res. **1032**: 104-110.

539 Hollins, J., Thambithurai, D., Koeck, B., Crespel, A., Bailey, D.M., Cooke, S.J., Jan
540 Lindström, J., Parsons, and K.J., Killen, S.S. 2018. A physiological perspective on
541 fisheries-induced evolution. Evol. Appl. doi: 10.1111/eva.12597
542 Huntingford, F., and Adams, C. 2005. Behavioural syndromes in farmed fish: implications
543 for production and welfare. Behav. **142**: 1207-1221.
544 Johnsson, J.I., Höjesjö, J., and Fleming, I.A. 2001. Behavioural and heart rate responses to
545 predation risk in wild and domesticated Atlantic salmon. Can. J. Fish. Aqu. Sci. **58**:
546 788-794.
547 Jørgensen, C., Ernande, B. and Fiksen, O. 2009. Size-selective fishing gear and life history
548 evolution in the Northeast Arctic cod. Evol. Appl.2:356-370.
549 Jørgensen, C. and Holt, R.E. 2013. Natural mortality: Its ecology, how it shapes fish life
550 histories, and why it may be increased by fishing. Journal of Sea Research 75:8-18.
551 Juette, T., Cucherousset, J. and Cote, J. 2014. Animal personality and the ecological impacts
552 of freshwater non-native species. Curr. Zool. 60:417-427.
553 Kekäläinen, J., Podgorniak, T., Puolakka, T., Hyvärinen, P. and Vainikka, A. 2014.
554 Individually assessed boldness predicts *Perca fluviatilis* behaviour in shoals, but is not
555 associated with the capture order or angling method. J. Fish Biol. **85**: 1603-1616.
556 Killen, S.S., Marras, S., Metcalfe, N.B., McKenzie, D.J. and Domenici, P. 2013.
557 Environmental stressors alter relationships between physiology and behaviour. Trends
558 Ecol. Evol. **28**: 651-658.
559 Klefoth, T., Skov, C., Krause, J., and Arlinghaus, R. 2012. The role of ecological context and
560 predation risk-stimuli in revealing the true picture about the genetic basis of boldness
561 evolution in fish. Behav. Ecol. Socio. **66**: 547-559.

562 Klefoth, T., Skov, C., Kuparinen, A., and Arlinghaus, R. 2017. Toward a mechanistic
563 understanding of vulnerability to hook-and-line fishing: Boldness as the basic target
564 of angling-induced selection. Evol. Appl.**10**: 994-1006.
565 Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster,
566 H., De Jong, I.C., Ruis, M.A.W., and Blokhuis, H.J. 1999. Coping styles in animals:
567 current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. **23**: 925-
568 935.
569 LeDoux, J. 2012. Rethinking the Emotional Brain. Neuron **73**: 653-676.
570 Lepage, O., Øverli, Ø., Petersson, E., Järvi, T., and Winberg, S. 2000. Differential stress
571 coping in wild and domesticated sea trout. Brain Behav. Evol. **56**: 259-268.
572 Louison, M.J., Adhikari, S., Stein, J.A., and Suski, C.D. 2017. Hormonal responsiveness to
573 stress is negatively associated with vulnerability to angling capture in fish. J. Exp.
574 Biol. **220**: 2529-2535.
575 Mezzera, M. and Largiadèr, and C.R. 2001. Evidence for selective angling of introduced trout
576 and their hybrids in a stocked brown trout population. J. Fish Biol. 59:287-301.
577 Moltesen, M., M. A. Vindas, S. Winberg, L. Ebbesson, M. de Lourdes Ruiz-Gomez, P. V.
578 Skov, T. Dabelsteen, Ø. Øverli, and E. Höglund. 2016. Cognitive appraisal of
579 aversive stimulus differs between individuals with contrasting stress coping styles;
580 evidences from selected rainbow trout (*Oncorhynchus mykiss*) strains. Behaviour **153**:
581 1567-1587.
582 Monk, C.T., and Arlinghaus, R. 2017a. Perch, *Perca fluviatilis*, spatial behaviour determines
583 vulnerability independent of angler skill in a whole-lake reality mining experiment.
584 Can. J. Fish. Aqu. Sci. doi: 10.1139/cjfas-2017-0029

585 Monk, C.T., and Arlinghaus, R. 2017b. Encountering a bait is necessary but insufficient to
586 explain individual variability in vulnerability to angling in two freshwater
587 benthivorous fish in the wild. PLoS ONE **12**:e0173989.
588 Moraga, A.D., Wilson, A.D.M., and Cooke, S.J. 2015. Does lure colour influence catch per
589 unit effort, fish capture size and hooking injury in angled largemouth bass? Fish. Res.
590 **172**: 1-6.
591 Palkovacs, E. P., Moritsch, M. M., Contolini, G. M., and Pelletier F. 2018. Ecology of
592 harvest-driven trait changes and implications for ecosystem management. Front. Ecol.
593 Environ. **16**:20-28.
594 Pankhurst, N.W. 2011. The endocrinology of stress in fish: an environmental perspective.
595 Gen. Comp. Endocrinol. **170**: 265-275.
596 Paul, A.J., Post, J.R., and Stelfox, J.D. 2003. Can Anglers Influence the Abundance of Native
597 and Nonnative Salmonids in a Stream from the Canadian Rocky Mountains? N. Am.
598 J. Fish. Manag. **23**: 109-119.
599 Pawson, M.G. 1991. Comparison of the performance of brown trout, *Salmo trutta* L., and
600 rainbow trout, *Oncorhynchus mykiss* (Walbaum), in a put-and-take fishery. Aquac.
601 Res. **22**: 247-257.
602 Philipp, D. P., Cooke, S. J., Claussen, J. E., Koppelman, J., Suski, C. D., and Burkett, D.
603 2009. Selection for vulnerability to angling in largemouth bass. Trans. Am. Fish. Soc.
604 **138**: 189-199.
605 Phillips, B. L., and Suarez, A. V. 2012. The role of behavioural variation in the invasion of
606 new areas. *In* Behavioural responses to a changing world: mechanisms &
607 consequences. *Edited by* U. Candolin and B. B. M. Wong. Oxford University Press,
608 Oxford, UK. pp. 190-200

609 Pigliucci, M., and Hayden, K. 2001. Phenotypic plasticity is the major determinant of
610 changes in phenotypic integration in Arabidopsis. New Phytol. **152**: 419-430.
611 Post, J.R., Sullivan, M., Cox, S., Lester, N.P., Walters, C.J., Parkinson, E.A., Paul, A.J.,
612 Jackson, L., and Shuter, B.J. 2002. Canada's recreational fisheries: the invisible
613 collapse? Fish **27**: 6-17.
614 Pottinger, T.G., Balm, P.H.M., and Pickering, A.D. 1995. Sexual maturity modifies the
615 responsiveness of the pituitary-interrenal axis to stress in male rainbow trout. Gen.
616 Comp. Endocrinol. **98**: 311-320.
617 Réale, D., Garant, D., Humphries, M.M., Bergeron, P., Careau, V., and Montiglio, P.-O.
618 2010. Personality and the emergence of the pace-of-life syndrome concept at the
619 population level. Philos. Trans. R. Soc. Lond., B, Biol. Sci. **365**: 4051-4063.
620 Redpath, T.D., Cooke, S.J., Suski, C.D., Arlinghaus, R., Couture, P., Wahl, D.H., and
621 Philipp, D.P. 2010. The metabolic and biochemical basis of vulnerability to
622 recreational angling after three generations of angling-induced selection in a teleost
623 fish. Can. J. Fish. Aqu. Sci. **67**: 1983-1992.
624 Ruane, N.M., Wendelaar Bonga, S.E., and Balm, P.H.M. 1999. Differences between rainbow
625 trout and brown trout in the regulation of the pituitary–interrenal axis and
626 physiological performance during confinement. Gen. Comp. Endocrinol. **115**: 210-
627 219.
628 Schreck, C. B., and L. Tort. 2016. The Concept of Stress in Fish. *In* Fish Physiology. *Edited*
629 *by* C. B. Schreck, L. Tort, A. P. Farrell, and C. J. Brauner. Academic Press. pp 1-34.
630 Singh, C., Oikonomou, G., Prober, D.A. 2015. Norepinephrine is required to promote
631 wakefulness and for hypocretin-induced arousal in zebrafish. eLife **4**: e07000.

632 Sundh, H., Calabrese, S., Jutfelt, F., Niklasson, L., Olsen, R.E., and Sundell, K. 2011.
633 Translocation of infectious pancreatic necrosis virus across the intestinal epithelium
634 of Atlantic salmon (*Salmo salar* L.). Aquac. **321**: 85-92.
635 Sundström, L.F., Petersson, E., Höjesjö, J., Johnsson, J.I., and Järvi, T. 2004. Hatchery
636 selection promotes boldness in newly hatched brown trout (*Salmo trutta*):
637 implications for dominance. Behav. Ecol. **15**: 192-198.
638 Sutter, D.A.H., Suski, C.D., Philipp, D.P., Klefoth, T., Wahl, D.H., Kersten, P., Cooke, S.J.,
639 and Arlinghaus, R. 2012. Recreational fishing selectively captures individuals with
640 the highest fitness potential. Proc. Natl. Acad. Sci. U.S.A.**109**: 20960-20965.
641 Tsuboi, JI., Morita, K., Klefoth, T., Endou, S., Arlinghaus, R. 2016. Behaviour-mediated
642 alteration of positively size-dependent vulnerability to angling in response to
643 historical fishing pressure in a freshwater salmonid. Can. J. Fish. Aqua. Sci. **73**:461-
644 468.
645 Uusi-Heikkilä, S., Wolter, C., Klefoth, T., and Arlinghaus, R. 2008. A behavioral perspective
646 on fishing-induced evolution. Trends Ecol. Evol. **23**: 419-421.
647 Uusi-Heikkilä, S. , Whiteley, A. R., Kuparinen, A. , Matsumura, S. , Venturelli, P. A.,
648 Wolter, C. , Slate, J. , Primmer, C. R., Meinelt, T. , Killen, S. S., Bierbach, D. ,
649 Polverino, G. , Ludwig, A. and Arlinghaus, R. 2015. The evolutionary legacy of size-
650 selective harvesting extends from genes to populations. Evol. Appl. **8**: 597-620.
651 Vainikka, A., Tammela, I., and Hyvärinen, P. 2016. Does boldness explain vulnerability to
652 angling in Eurasian perch *Perca fluviatilis*? Curr. Zool. **62**: 109-115.
653 Wendelaar Bonga, S. 1997. The stress response in fish. Physiol. Rev. **77**: 591-625.
654 Wilson, A.D.M., Brownscombe, J.W., Sullivan, B., Jain-Schlaepfer, S., and Cooke, S.J. 2015.
655 Does angling technique selectively target fishes based on their behavioural type?
656 PLoS ONE **10**: e0135848.

657 Winberg, S., Øverli, Ø., and Lepage, O. 2001. Suppression of aggression in rainbow trout
658 (*Oncorhynchus mykiss*) by dietary L-tryptophan. J. Exp. Biol. **204**: 38-67.
659 Winberg, S., and Thörnqvist, P.-O. 2016. Role of brain serotonin in modulating fish behavior.
660 Curr. Zool. **62**: 317-323.
661 Wise, R.A. 2004. Dopamine, learning and motivation. Nat. Rev. Neurosci. **5**: 483-494.
662 Young, G. 1986. Cortisol secretion in vitro by the interrenal of coho salmon (*Oncorhynchus*
663 *kisutch*) during smoltification: relationship with plasma thyroxine and plasma cortisol.
664 Gen. Comp. Endocrinol. **63**: 191-200.
665 Závorka, L., Koeck, B., Cucherousset, J., Brijs, J., Näslund, J., Aldvén, D., Höjesjö, J.,
666 Fleming, I.A., and Johnsson, J.I. 2017. Co-existence with non-native brook trout
667 breaks down the integration of phenotypic traits in brown trout parr. Funct. Ecol. **31**:
668 1582–1591.
669 Závorka, L., Lang, I., Raffard, A., Evangelista, C., Britton, J.R., Olden, J.D., and
670 Cucherousset, J. (in press). Importance of harvest-driven trait changes for invasive
671 species management. Front Ecol Environ **FEE18-0014.**

Tables

Table 1. Cox-proportional hazards regression model comparing the time until first capture and the probability of capture of rainbow and brown trout, with β the corresponding hazard coefficient and e^{β} the hazard probability. The number of events refers to the total number of captured individuals.

	β	e^{β}	se(β)	z	p-value
Species (Rainbow trout)	0.8728	2.3935	0.252	3.454	0.0005
n= 93, number of events= 73					
Likelihood ratio test = 12.76 on 1 df, p < 0.001					

678 **Table 2.** Cox-proportional hazards regression model examining the time until first capture
679 and the probability of capture of rainbow trout in relationship to individual pond activity (i.e.
680 number of detections) and plasma cortisol levels (after exposure to a standardized
681 experimental stressor), with β the corresponding hazard coefficient and e^{β} the hazard
682 probability. The number of events refers to the number of individuals captured by angling
683 during angling experiment 1, in pond 1 and 3 (i.e. under high intensity angling treatment).

	β	e^{β}	se(β)	z	p-value
Detections	0.1827	1.2005	0.074	2.346	0.019
Cortisol	-0.0048	0.9951	0.003	-2.083	0.037
n = 47, number of events = 44					
Likelihood ratio test = 11.99 on 2 df, p = 0.002					

684

685

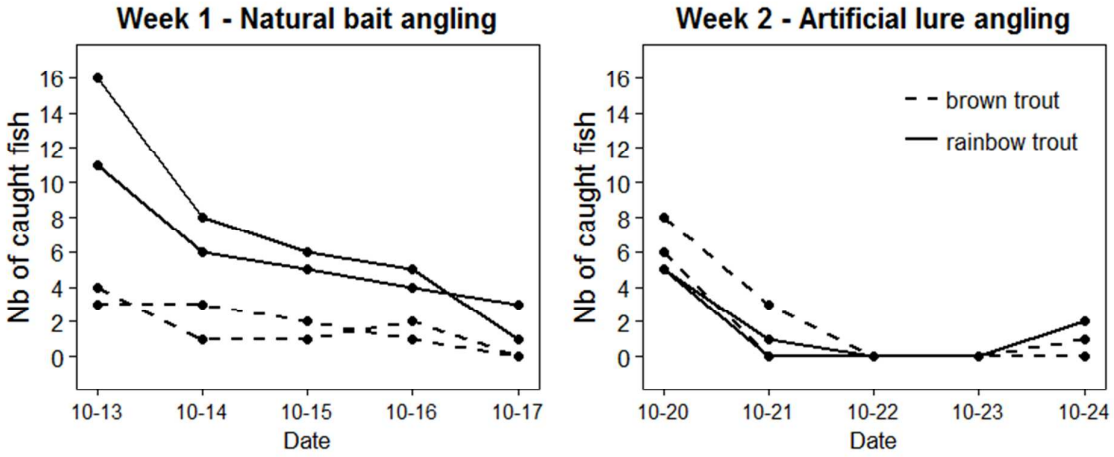
686 **Table 3.** Cox-proportional hazards regression model examining the time until first capture
687 and the probability of capture of brown trout in relationship to individual pond activity (i.e.
688 number of detections) and plasma cortisol levels (after exposure to a standardized
689 experimental stressor), with β the corresponding hazard coefficient and e^{β} the hazard
690 probability. The number of events refers to the number of individuals captured by angling
691 during angling experiment 1, in pond 1 and 3 (i.e. under high intensity angling treatment).

	β	e^{β}	se(β)	z	p-value
Detections	-0.0064	0.9936	0.103	-0.069	0.945
Cortisol	0.0041	1.0041	0.004	0.959	0.338
n = 37, number of events = 24					
Likelihood ratio test = 0.82 on 2 df, p = 0.663					

692

693

694 Figures

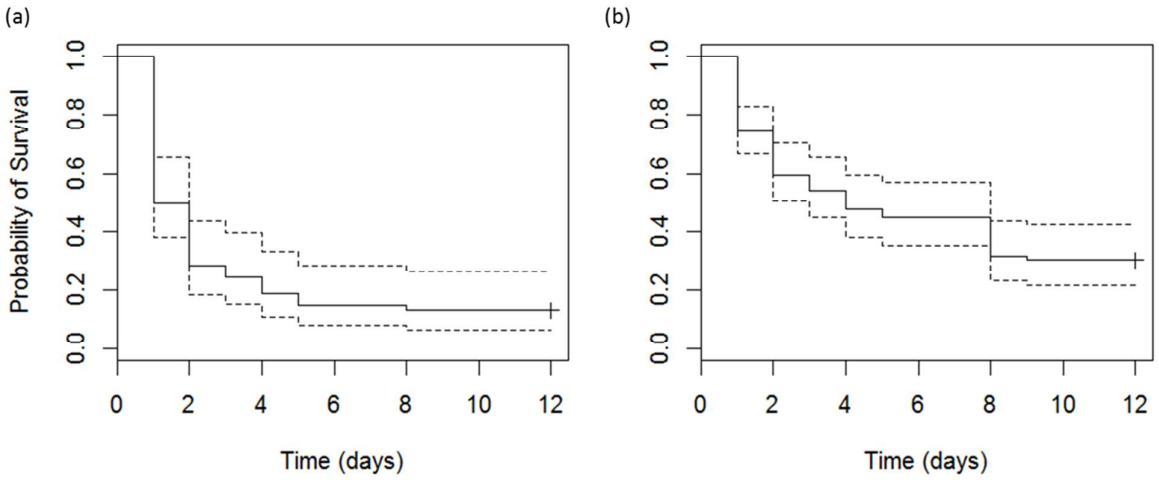


695

696 **Figure 1.** Number of captured fish per angling day for successive bait and lure angling

697 (experiment 1) in pond 1 and 3.

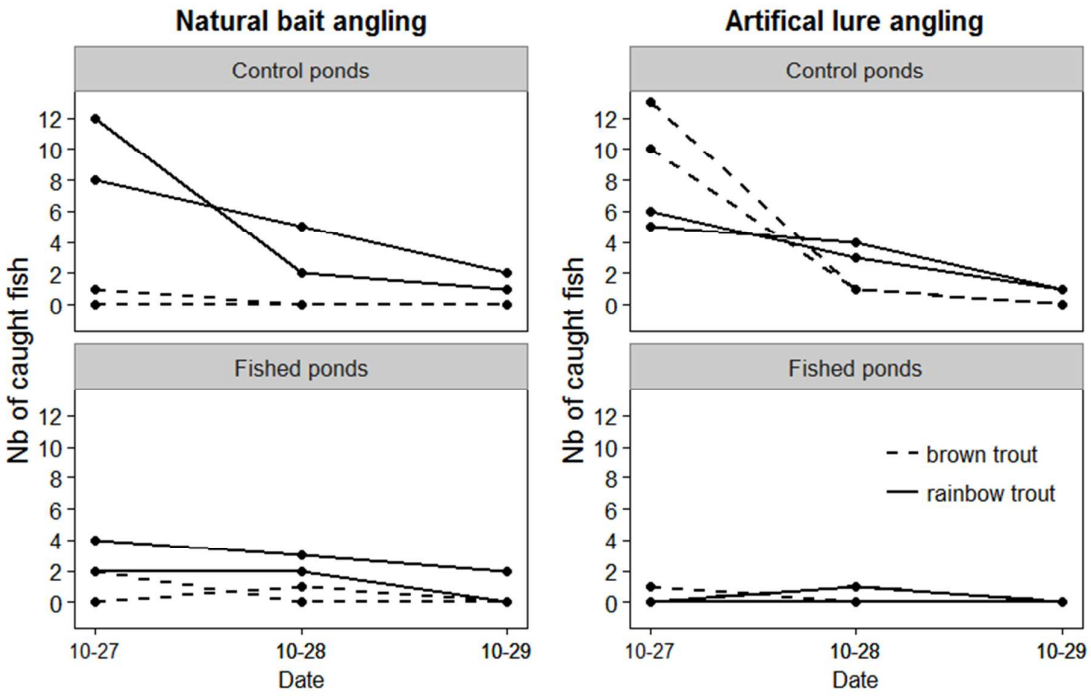
698



699

700 **Figure 2.** Survival curves (i.e. probability of remaining uncaptured over time) of rainbow
701 trout (a) and brown trout (b) during the first angling experiment in ponds 1 and 3 (i.e. under
702 high intensity angling treatment). Dashed lines correspond to a 95% confidence envelop
703 around the survival function. (day 1 to 5: natural bait angling, day 8 to 12: artificial lure
704 angling).

705



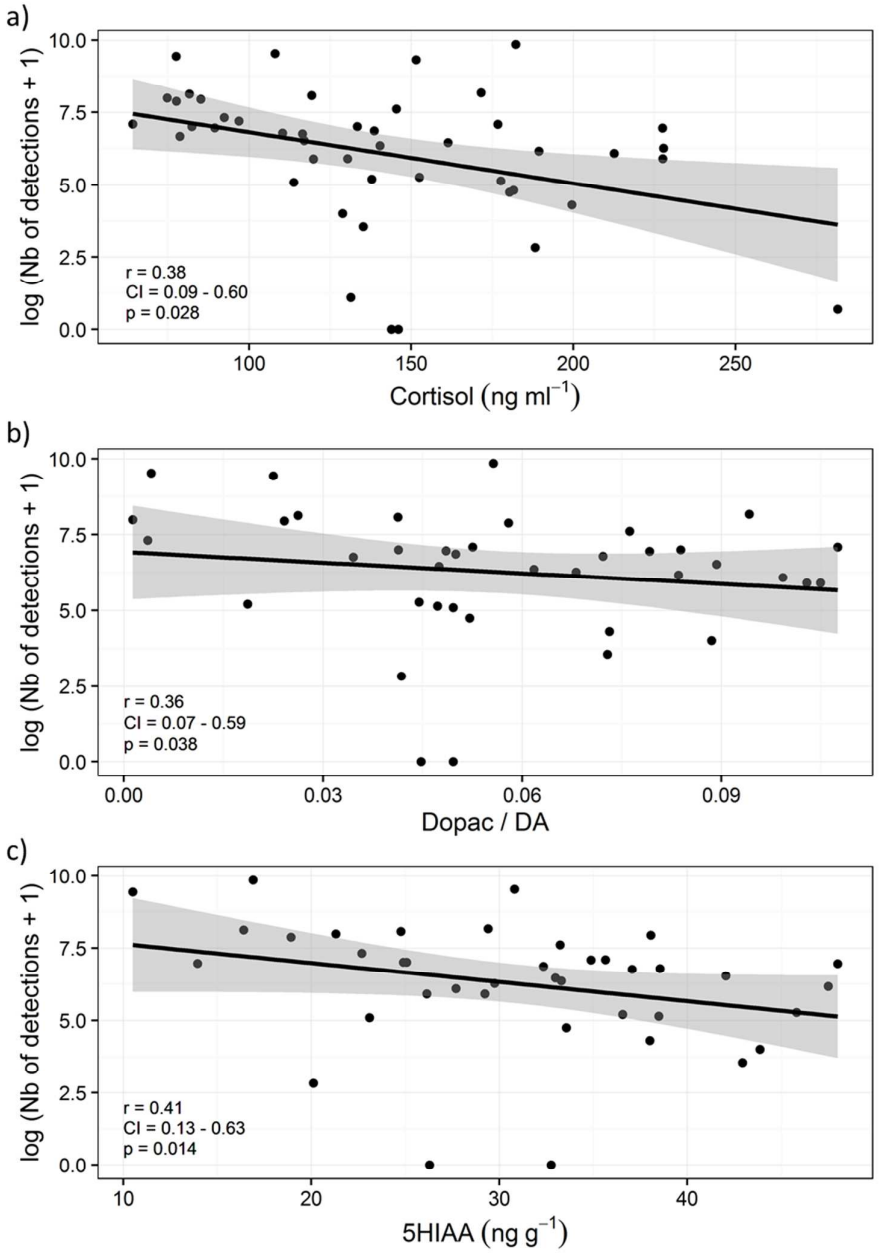
706

707 **Figure 3.** Number of captured fish per angling day for simultaneous bait and lure angling

708 (experiment 2) in previously unfished control ponds (ponds 2 and 4: low angling intensity)

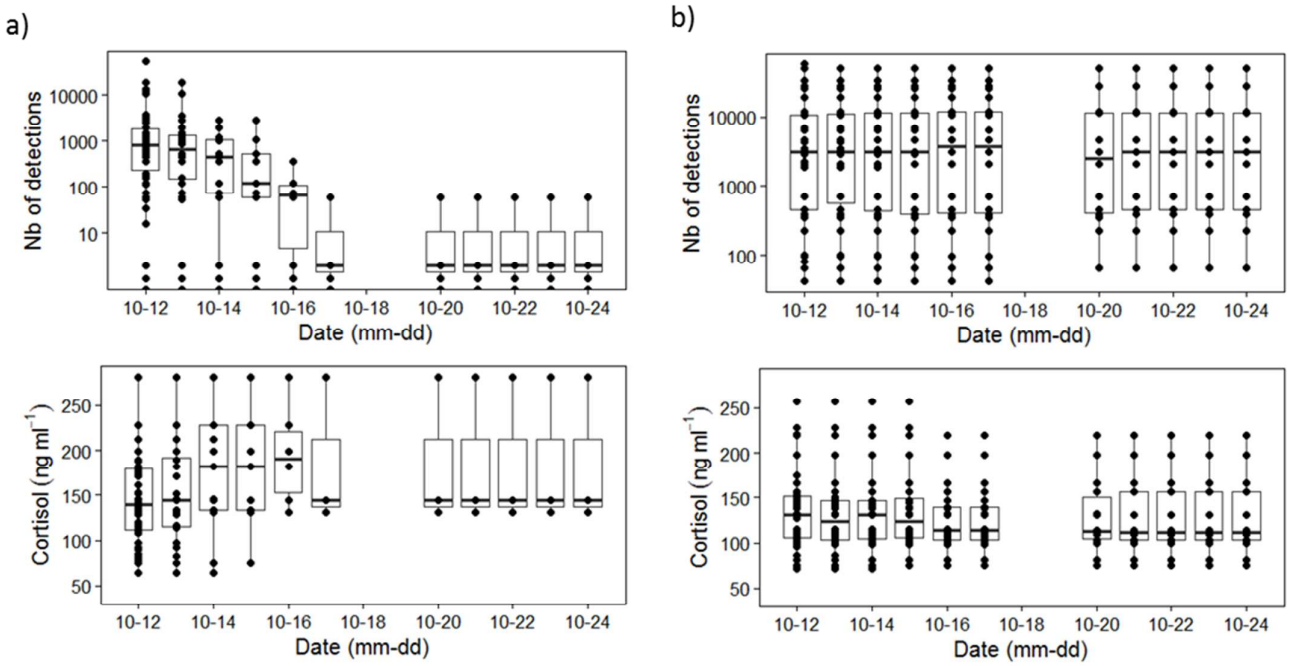
709 and previously fished ponds (ponds 1 and 3: high angling intensity).

710



711

712 **Figure 4.** Correlation plots for rainbow trout from the high intensity angling treatment
713 between pond activity (log transformed number of detections) and plasma cortisol, dopamine
714 turnover (DOPAC / DA) and serotonin metabolite 5-HIAA levels after exposure to a
715 standardized experimental stressor. Pearson's r correlation coefficient, confidence intervals
716 CI and p-values are displayed. A linear model was fitted (solid line) and a 95% confidence
717 interval estimated (grey area).



718

719 **Figure 5.** Average pond activity and cortisol level (after exposure to a standardized
720 experimental stressor) of the remaining uncaptured rainbow trout (a) and brown trout (b) over
721 angling days of experiment 1 and high intensity angling treatment. Box-plots of the first
722 angling day (October 12th) represent the average pond activity and cortisol level of rainbow
723 and brown trout from the high intensity angling treatment (pond 1 and 3). After the first
724 capture of an individual, its' corresponding activity or cortisol level value is removed from
725 the dataset and the average value recalculated for the remaining uncaptured fish.

726

Appendix: Angling selects against stress-resilient and active phenotypes

Barbara Koeck, Libor Závorka, David Aldvén, Joacim Näslund, Robert Arlinghaus, Per-Ove Thörnqvist, Svante Winberg, Björn Thrandur Björnsson, Jörgen I. Johnsson

A1. Experimental pond setup

Two full-scale replicated angling experiments were successively carried out in four semi-controlled mesocosm ponds (area = 30 × 24 = 720 m^2, depth = 2 m) belonging to the Swedish anglers association *Sportfiskarna* in Gothenburg, Sweden (57.693°N, 12.037°E). Prior to the experiments, vegetation and fish were removed from all ponds to create comparable conditions. Ponds were supplied with unfiltered lake water (Lake *Delsjön*) containing natural food supply for trout. Each pond was stocked with size-matched rainbow (298 ± 19 mm) and brown trout (281 ± 15 mm) in equal densities (25 individuals per species and pond). Both trout species originated from the *Källefalls* hatchery and were reared under comparable conditions (: http://www.hokensas.se/en/fish-farming/kallefall/). The brown trout were F1 offspring from wild parents captured in the near-by Lake *Vättern*. The rainbow trout were of a domesticated strain, now bred since 1997 within the hatchery and used exclusively for stocking for angling in Swedish waters.

A2. Angling gear and design

Two commonly used angling techniques were used for the angling experiments; natural shrimp baits and spinnerbait lures, to be representative of angler habits. Natural bait angling was carried out using the shrimp on a barbless hook (size 6), tackled beneath a floater. Anglers were fishing passively with the shrimp bait by casting it and retrieving it slowly, while lure angling was fished actively using a spinnerbait (Myrans WIPP Yellow/White, 10 g; Sportsystem i Jönköping AB, Huskvarna), which was cast and retrieved at a fast speed. Despite the fact that angling is considered a generally passive fishing method, in order to discriminate both angling methods in the present study, we refer to lure angling as an active method and to bait angling as a passive angling method. With the exception of these two types of terminal tackles, identical angling equipment was used for all angling experiments

(Abu Garcia rod: 7 ft, power 10-40 g; braided line: resistance 10 lb; "Impact" haspel spinning reel: size 3000).

Angling experiments were carried out by experienced volunteer anglers, who were instructed to the protocol of the experiments to reduce bias associated to fish handling after capture. Hooked fish were landed with a knotless net and unhooked by fishermen using a plier designed for that specific purpose. Fish were then kept in a holding tank (L×W×D; 90 × 90 × 40 cm) at the side of the pond until returned to their pond at the end of the fishing event. A fish could thus be captured only once per fishing event, but recaptured at each new event. Unless conditions required so, handling of fish with bare hands was avoided to limit mucus abrasion. Date, time, pond and PIT-tag number (hand-held PIT tag reader; BTS-ID, Helsingborg, Sweden) were recorded.

In angling experiment 1, angling was practiced in only pond 1 and pond 3 of the four experimental ponds, and consisted of five days of natural bait angling followed by five days of lure angling, with a two days interval without fishing in between (Fig. 1a). In angling experiment 2, angling was performed in all four ponds with ponds 1 and 3 previously exposed to angling and ponds 2 and 4 containing fish naïve to angling (Fig. 1b). It consisted of three angling events with simultaneous bait and lure angling to verify the results from the species-specific selectivity of angling technique from experiment 1, while excluding the temporal effect of a successive use of angling technique. To spread out the angling effort evenly, anglers were assigned to defined fishing zones within in each pond. Every tenth minute anglers changed fishing zone and/or angling gear (i.e. artificial lure or natural bait) according to a randomization schedule to control for bias in fishing skills of anglers, site preference and gear effect. At the end of the two angling experiments, ponds 1 and 3 had

each received a total fishing effort of 36 hours (high angling intensity treatment) and pond 2 and 4 had received only 6 hours of angling (low angling intensity treatment).

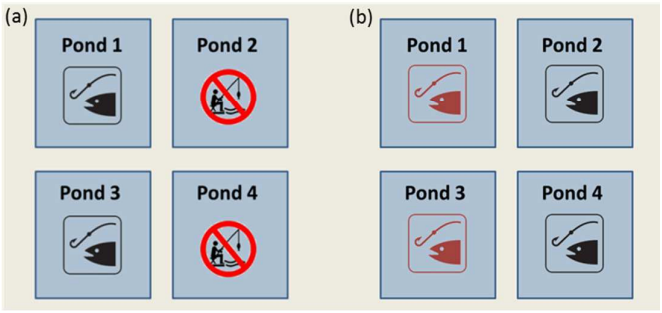


Figure A1. Experimental treatments in the four mesocosm ponds for (a) the first angling experiment, and (b) the second angling experiment, where fish in pond 1 and 3 had already experienced angling and fish in pond 2 and 4 were naïve to angling.

A3. Behavioral scoring

The scoring consisted of recording activity of individual fish in an open-field test in white barren tanks (L×W×D; 90 × 90 × 40 cm) using a video-camera positioned above the tanks during 20 min following 10 min of acclimation. The automated image analysis software LoliTrack (4.0, Loligo Systems ApS) was used to analyse the video records and extract an activity proxy based on the cumulated distance moved during the 20-min trial by each individual. Fish from a same holding tank were scored at the same time to standardize fish handling and to reduce any bias linked to handling stress.

A4. Correlations between individual activity and physiological stress responses

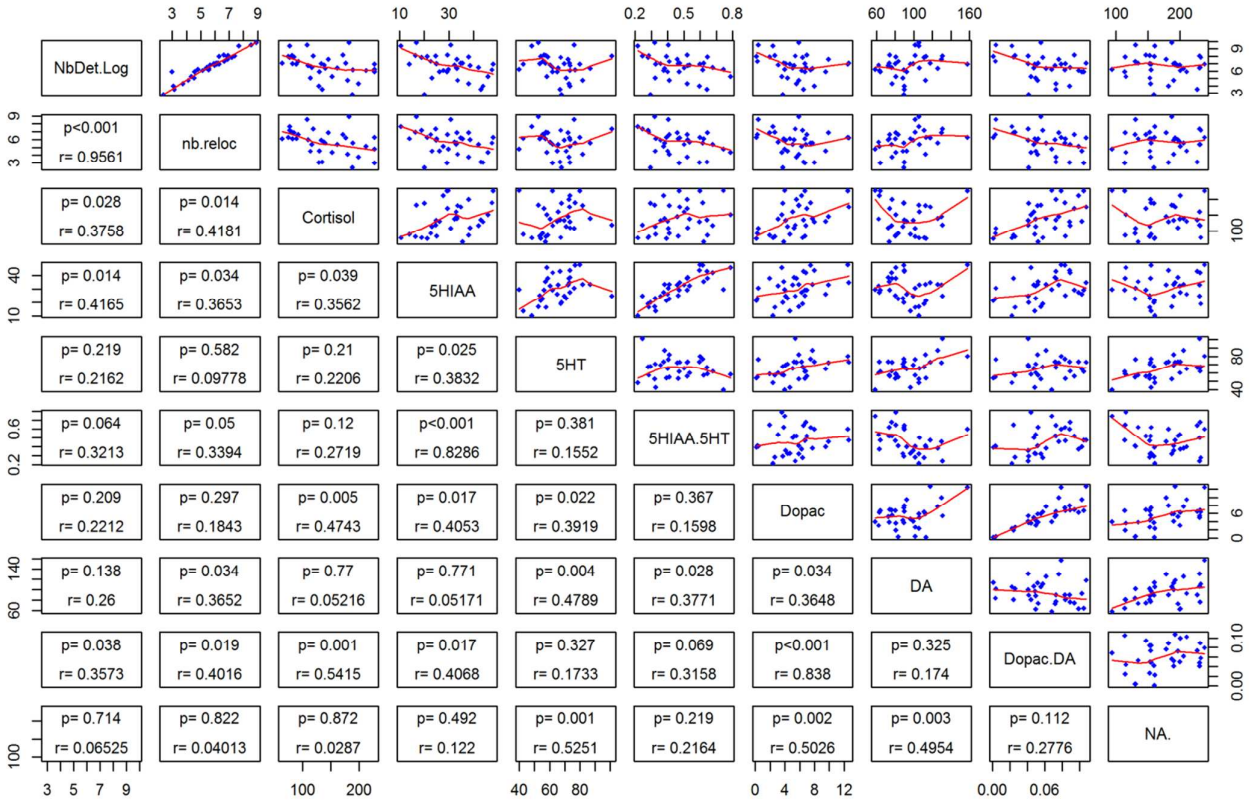


Figure A2. Pearson's correlation matrix for rainbow trout from the high intensity angling treatment between pond activity (NbDet.Log: log transformed number of total detections; nb.reloc: number of relocations between antennas), plasma cortisol (ng ml^{-1}), neurotransmitters (ng g^{-1}) and neurotransmitter activity levels (Dopac.DA: dopaminergic activity; 5-HIAA.5-HT: serotonergic activity) after exposure to a standardized experimental stressor. Pearson's r correlation coefficient and adjusted p-values are given in the lower half of the matrix and correlation plots in the upper part of the matrix.

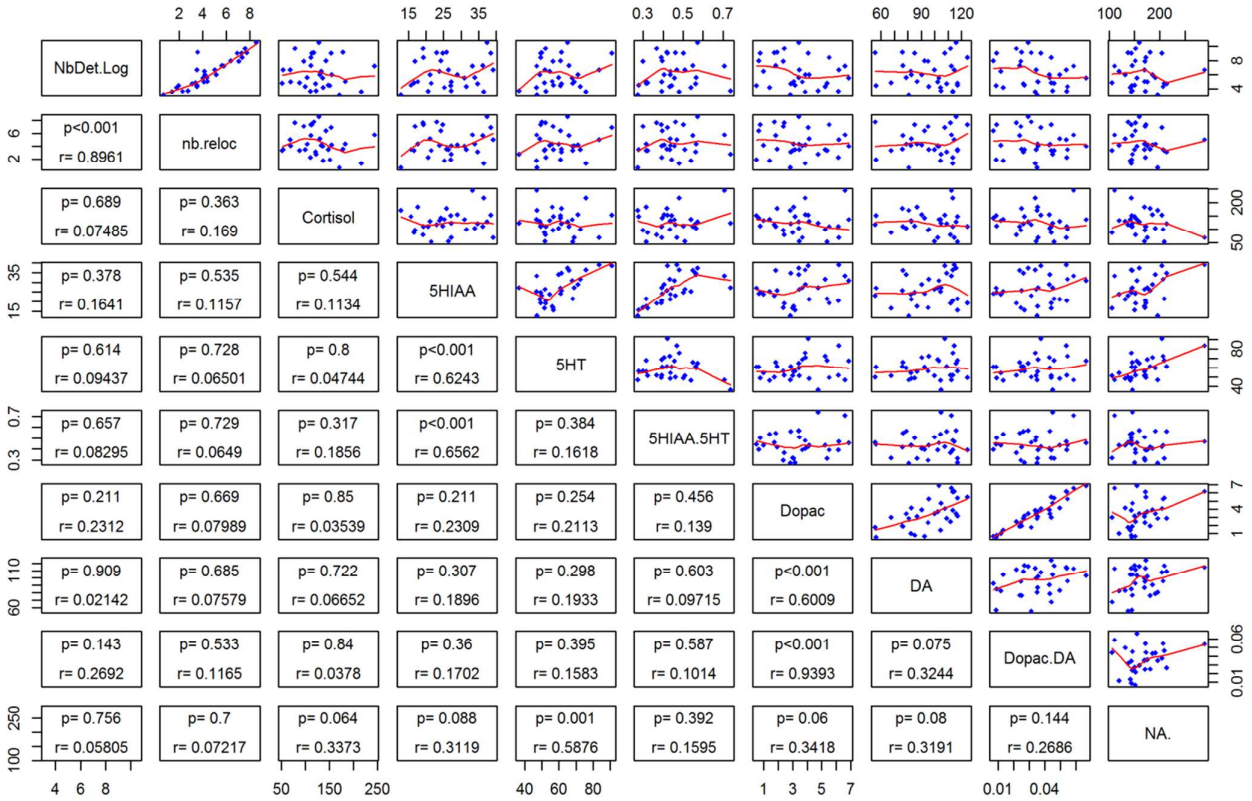


Figure A3. Pearson's correlation matrix for rainbow trout from the low intensity angling treatment between pond activity (NbDet.Log: log transformed number of total detections; nb.reloc: number of relocations between antennas), plasma cortisol (ng ml^{-1}), neurotransmitters (ng g^{-1}) and neurotransmitter activity levels (Dopac.DA: dopaminergic activity; 5-HIAA.5-HT: serotonergic activity) after exposure to a standardized experimental stressor.

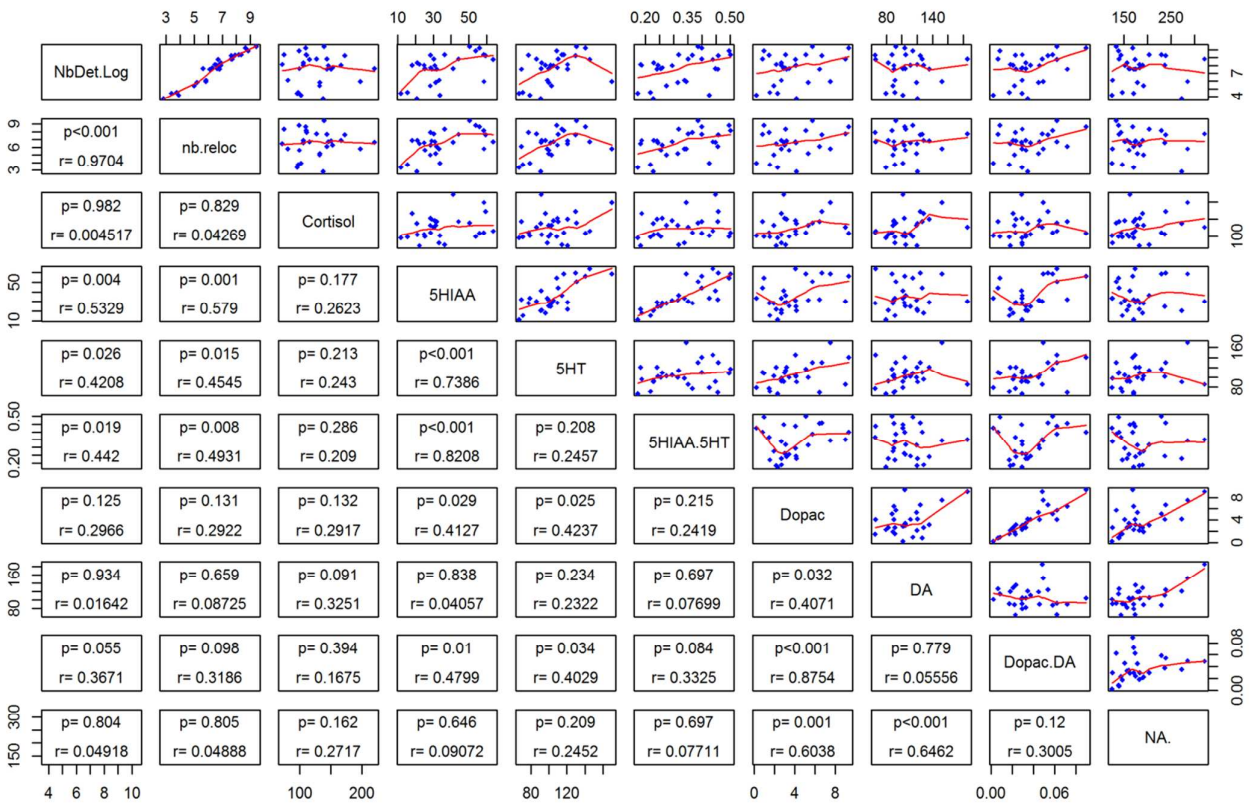


Figure A4. Pearson's correlation matrix for brown trout from the high intensity angling treatment between pond activity (NbDet.Log: log transformed number of total detections; nb.reloc: number of relocations between antennas), plasma cortisol (ng ml^{-1}), neurotransmitters (ng g^{-1}) and neurotransmitter activity levels (Dopac.DA: dopaminergic activity; 5-HIAA.5-HT: serotonergic activity) after exposure to a standardized experimental stressor.

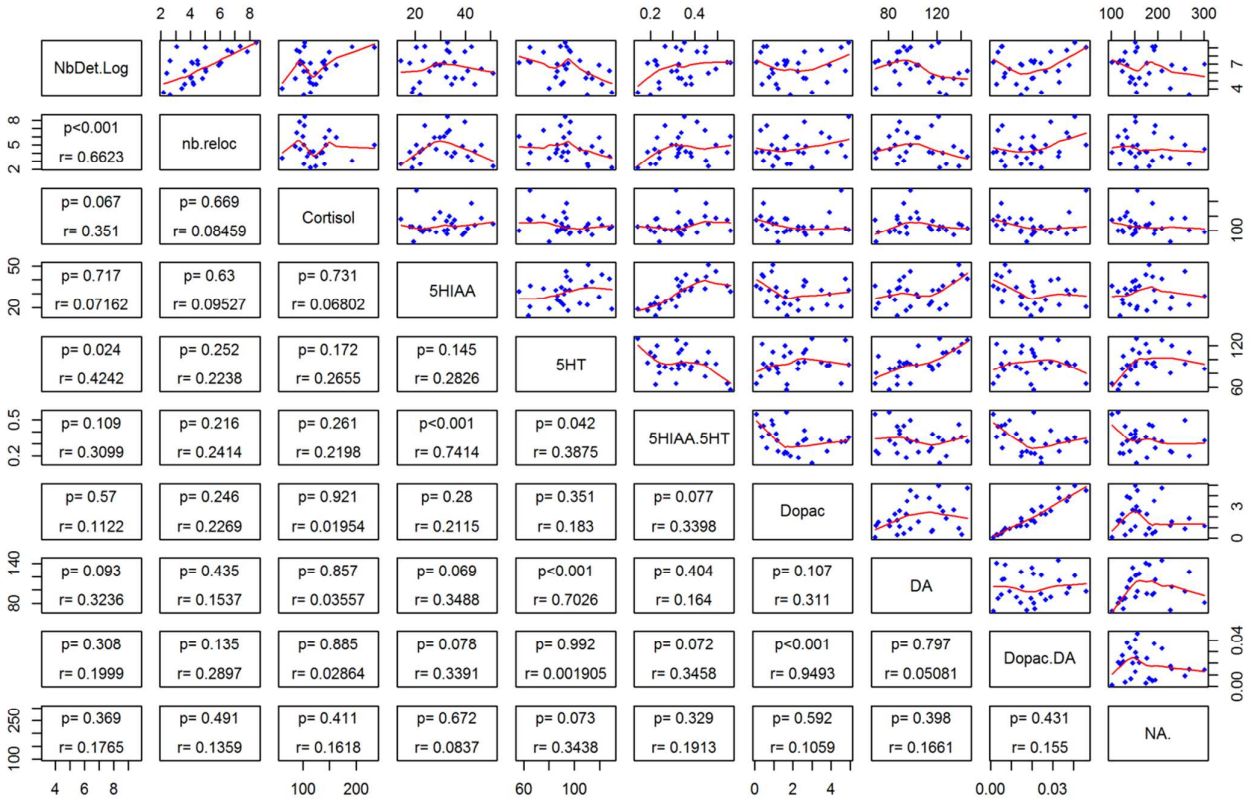


Figure A5. Pearson's correlation matrix for brown trout from the low intensity angling treatment between pond activity (NbDet.Log: log transformed number of total detections; nb.reloc: number of relocations between antennas), plasma cortisol (ng ml^{-1}), neurotransmitters (ng g^{-1}) and neurotransmitter activity levels (Dopac.DA: dopaminergic activity; 5-HIAA.5-HT: serotonergic activity) after exposure to a standardized experimental stressor.